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TRANSFER OF RADIONUCLIDES FROM THE ENVIRONMENT TO HUMAN MILK PHASE 2 - FEASIBILITY STUDY

by

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TRANSFER OF RADIONUCLIDES FROM THE ENVIRONMENT
TO HUMAN MILK - PHASE 2: FEASIBILITY STUDY

ABSTRACT

This report gives detailed procedures for :

- collecting, storing and shipping human milk samples;
- complete decomposition of human milk samples by nitric acid-perchloric acid oxidation;
- sequential separation and determinations of lead 210, radium 226, thorium 230, natural thorium and natural uranium from decomposed human milk solutions.

This sequential separation method is capable of detecting 0.025 Bq of radium 226, thorium 230 and thorium 232 and 0.05 Bq of lead 210. Recoveries are approximately 70% for radium 226 and 90% for thorium 230, thorium 232 and lead 210.

This report also outlines a proposed field study program.

RÉSUMÉ

Le présent rapport indique des méthodes détaillées pour :

- le rassemblement, l'entreposage et l'expédition des échantillons de lait maternel;
- la décomposition totale des échantillons de lait maternel par oxydation à l'acide nitrique et à l'acide perchlorique;
- la séparation séquentielle et la détermination subséquente de la teneur en plomb 210, en radium 226, en thorium 230, en thorium et en uranium naturels dans les solutions de lait maternel décomposées.

La méthode de séparation séquentielle peut déceler 0,025 Bq de radium 226, de thorium 230 et de thorium 232, ainsi que 0,05 Bq de plomb 210. Les recouvrements sont approximativement de 70 pour 100 pour le radium 226 et de 90 pour 100 pour le thorium 230, le thorium 232 et le plomb 210.

Le rapport donne aussi les grandes lignes d'un projet de programme d'études sur place.

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RECOMMENDATION

Concern has been expressed regarding the contribution of human milk to the total radiological dose commitment of a breast-feeding infant. In Phase I of this project, scientific literature has been reviewed with the objective of obtaining information pertaining to the transfer of radioactivity from the environment to human milk. No information has yet been found for the naturally occurring radioisotopes of the uranium and thorium decay series. Phase I also reports that there is no simple method for predicting such a transfer. It recommends that before a field survey is initiated, several considerations should be assessed. These include the development of appropriate analytical methods for the activity levels expected in human milk and standardized sampling protocol.

Phase 2 of this project was carried out to develop the sampling and analysis techniques for determining lead-210, radium-226, thorium-230, natural thorium and natural uranium in human milk. The results presented in this report show that low levels of these radioisotopes can be analysed accurately and precisely. In addition this report proposes a detailed plan to carry out a field survey to determine these radioisotopes in human milk.

As a result of the findings presented in this report, it is recommended that a field study be initiated. The justification for this recommendation is as follows:

(1) No information on this subject is currently available in literature.

(2) As pointed out in Phase I, there is no simple method for predicting an element's transfer behaviour in the food chain, the only means available for bridging the information gap is actually to make the measurement.

(3) The analysis techniques are available for making such measurements at level of precision and accuracy that will allow meaningful comparisons to be made between geographical regions, age and diet of mother and other factors.

(4) The Canadian Shield is known to be high in uranium deposits, particularly in northern Saskatchewan. Since the Canadian native Indian and Eskimo women use ground water and eat animals feeding in these uranium rich regions, it is possible that high radioactivity levels could be found in the milk of nursing mothers. For example, there are many references in scientific literature which point out that abnormally high levels of lead-210 and polonium-210 are found in reindeer of the north.

(5) It is known that radium-226 is a "bone seeker". Therefore anomalous levels of Ra-226 in mother's milk would be expected to give rise to anomalous levels of Ra-226 in the bones of the infants.

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1. INTRODUCTION

In order to study the movement of radionuclides from nuclear facilities to and through the environment and to estimate the doses that may be received by members of the public, Atomic Energy Control Board has sponsored an investigation into the significance of the pathway which involves the transfer of radionuclides to a child through human milk. Phase I of this investigation has been completed recently by Eamon[1]. The report provides no relevant scientific information pertaining to the radionuclides of the uranium and thorium decay series present in human milk. It recommends that, prior to launching a field study program to collect and analyse human milk samples for radionuclides, suitable analytical techniques should be established.

The objectives and scope of this Phase 2 study are:

(1) To evaluate and test analytical techniques and to select the most suitable one for the determination of radionuclides in human milk.

This study has established the analytical techniques required which include the methodology for the complete decomposition of human milk samples, sequential separation of the radionuclides and radiometric identification and quantification of these radionuclides. The radionuclides include radium-226 (Ra-226), lead-210 (Pb-210), thorium-228 (Th-228), thorium-230 (Th-230), thorium-232 (Th-232 or natural thorium), and natural uranium.

(2) To establish human milk sampling techniques.

This report contains detailed procedures for collecting, storing and shipping of human milk samples.

(3) To prepare a plan for implementing the field study program.

This report recommends a detailed plan which includes the study schedule and cost estimates.

2. DEVELOPMENT OF METHODOLOGY FOR HUMAN MILK

2.1 HUMAN MILK SAMPLING

Human milk can be collected either by manual expression or by the use of a breast pump. Manual expression is encouraged as it is the least traumatic for the donors. Special manual expression technique is available in pamphlets or can be taught by nurses. Breast pumps are available in both manual and electric types. For donors who are unable to master the technique of manual expression, the use of a manual pump is suggested. There are many varieties available and the best performing pumps appear to be those based on the principle of the cyclinder bicycle pump. Electric pumps are very expensive although effective. The use of pumps for milk expression might cause tissue trauma due to the suction action of the nipple and the potential for bacterial growth. A 1981 survey of Canadian mothers who donated milk to milk banks indicates that 74% preferred manual expression[2].

Human milk can be stored safely in the frozen state, both raw and pasteurized. In both cases, no preservative is required. They can be stored in glass bottles, sterile disposable bags and plastic containers. Human milk storage time in Canadian milk banks varied from two weeks to two years with a mean storage time of nine months. It is recommended that storage time in refrigerator freezer compartments be kept under three months and in a deep freezer up to one year.

With the ever expandng overnight courier services across the country shipping from a sampling site to a laboratory is quite simple. Frozen milk samples can be shipped simply by packaging in an inexpensive foam cooler with either ice cubes or gel freezer packs. For isolated sampling sites, packaging with dry ice will extend the transit time to three or four days.

2.2 DECOMPOSITION OF HUMAN MILK SAMPLES

There are various methods for sample decomposition[3-16]. The choice depends on the nature of the sample and the analytical work which is to follow.

Human milk, like bovine milk, is composed of approximately 87% water, 12% organics, and less than 1% inorganics. The major organic constituents are protein, lipid and carbohydrate while the major inorganic constituents are calcium, magnesium, sodium, potassium, phosphorus, and chloride. The main differences are in the higher protein and inorganics content of bovine milk and the greater carbohydrate content of human milk[17,18]. Table 1 lists the average composition of human and bovine milk. Milk is a combination of three physical states, namely aqueous solution, emulsion, and colloidal suspension. In the aqueous solution portion of milk, fat occurs in emulsified form while a number of other constituents such as lactose, water soluble vitamins, and minerals are dissolved. The proteins are dispersed in a colloidal state. In addition, a number of compounds such as phospholipids, fat soluble vitamins and sterols are held in the fat globules. As to the radionuclides of interest, there is no information available in literature suggesting with which physical state(s) they are associated.

For the analysis of radionuclides of uranium and thorium decay series, the sample matrix must be completely decomposed and the element sought must be converted reliably to the proper ionic form[16]. In the case of milk samples, all organic constituents must be completely destroyed with only the inorganic constituents left in an aqueous solution. Two commonly used methods which can decompose organic matters are dry ashing and wet ashing.

Dry ashing of samples containing organic matter involves the evaporation of water content initially at 100°C, ashing of

TABLE 1. Average Composition of Human & Bovine Milk[17,18]

CONSTITUENTS	HUMAN		BOVINE	
	%	mg/L	%	mg/L
Water	87.5		87.	
Protein	1.0-1.5		3.0-4.0	
Lipid	3.0-4.0		3.5-5.0	
Carbohydrate	7.0-7.5		4.5-5.0	
Ash	0.2		0.75	
Ca		330		1260
Mg		45		130
Na		105		400
K		470		1550
P		145		870
Cl		330		1020

organics at 100 - 325°C, and finally destroying carbon at 500°C[19]. However, this method has several disadvantges. The most serious one is the potential loss of lead[20] and polonium[21] by volatilization and by plating onto metallic containers (if used). During ashing, two occurrences can increase the ashing temperatures over the maximum required. One of these is the ignition of the sample if large amount of lipid is present. The other is the glowing of the carbonaceous material due to oxidation of carbon. A second disadvantage is that many tervalent and higher valent elements form refractory oxides and carbides while being treated under dry ashing conditions. Vigorous treatment of the ash is then required to ensure the complete conversion of these elements to the proper ionic state[16]. Thorium is one of those elements. Cross contamination and loss of sample due to splashing is very difficult to prevent since it is done inside a muffle furnace. Additional foreign impurities are normally introduced into the resulting ashes due to corrosion of metallic containers or etching of silica and porcelain containers.

Wet ashing of sample containing organic matter is usually done under oxidixing conditions in the presence of acid such as nitric acid, sulfuric acid or perchloric acid. This method has several advantages. Firstly, since only acid is used, no cations, other than hydrogen are added. Also the excess acid can be removed with ease by volatilization. Secondly, the ashing temperature is relatively low, e.g. from room temperature up to the boiling point of the acid used. Finally, only glasswares or teflonwares are needed.

Based on the above comparison, the wet ashing method was chosen for the decomposition of milk samples. Preliminary testing was conducted using raw bovine milk samples. Ashing with sulfuric acid caused extensive charring of the sample due to the strong dehydration power of sulfuric acid on the organic constituents. The resulting black mixture could not be heated above 80°C, without serious splashing. Ashing with nitric acid proceeded

smoothly but incompletely due to the moderate oxidizing power of nitric acid. Addition of perchloric acid to the nitric acid completed the oxidation of the resilient organic molecules, due to the very strong oxidizing power of perchloric acid. A pure white ash residue was obtained which dissolved completely in dilute hydrochloric acid. Solutions from the decomposition of 250 ml aliquots of human milk and bovine milk were analysed for the presence of organic matter. Less than 10 mg were found in all cases.

2.3 SEPARATION OF RADIONUCLIDES

In routine radionuclide analysis, a separate aliquot or subsample, is used for each radionuclide. However, when limited sample and/or low activities are expected, sequential separation of all radionuclides from the entire sample is not only essential, but also maximizes the sensitivity of their determination. Two sequential separation schemes which include radionuclides such as Ra-226, Pb-210, Th-230, Th-232, and uranium, have been reported. One was developed by Koide and Bruland[25] (Scheme 1) for sea water and sediments and the other was developed by Sill[26] (Scheme 2) for uranium ores, dusts, and mill tailings. Since all milk samples, after decomposition, contain only the inorganic constituents in aqueous solutions, these schemes should also be applicable for milk samples. These schemes, with some modifications, are shown schematically in Figure 1 and Figure 2.

2.4 DETERMINATION OF RADIONUCLIDES

2.4.1 Radium-226

Radium-226 can be determined by five techniques: namely, gross alpha measurement[27-29], radon emanation[20,30-40], alpha spectroscopy[41-47], gamma spectroscopy[48-50] and coincidence spectroscopy[51-53]. Gross alpha measurement gives total radium.

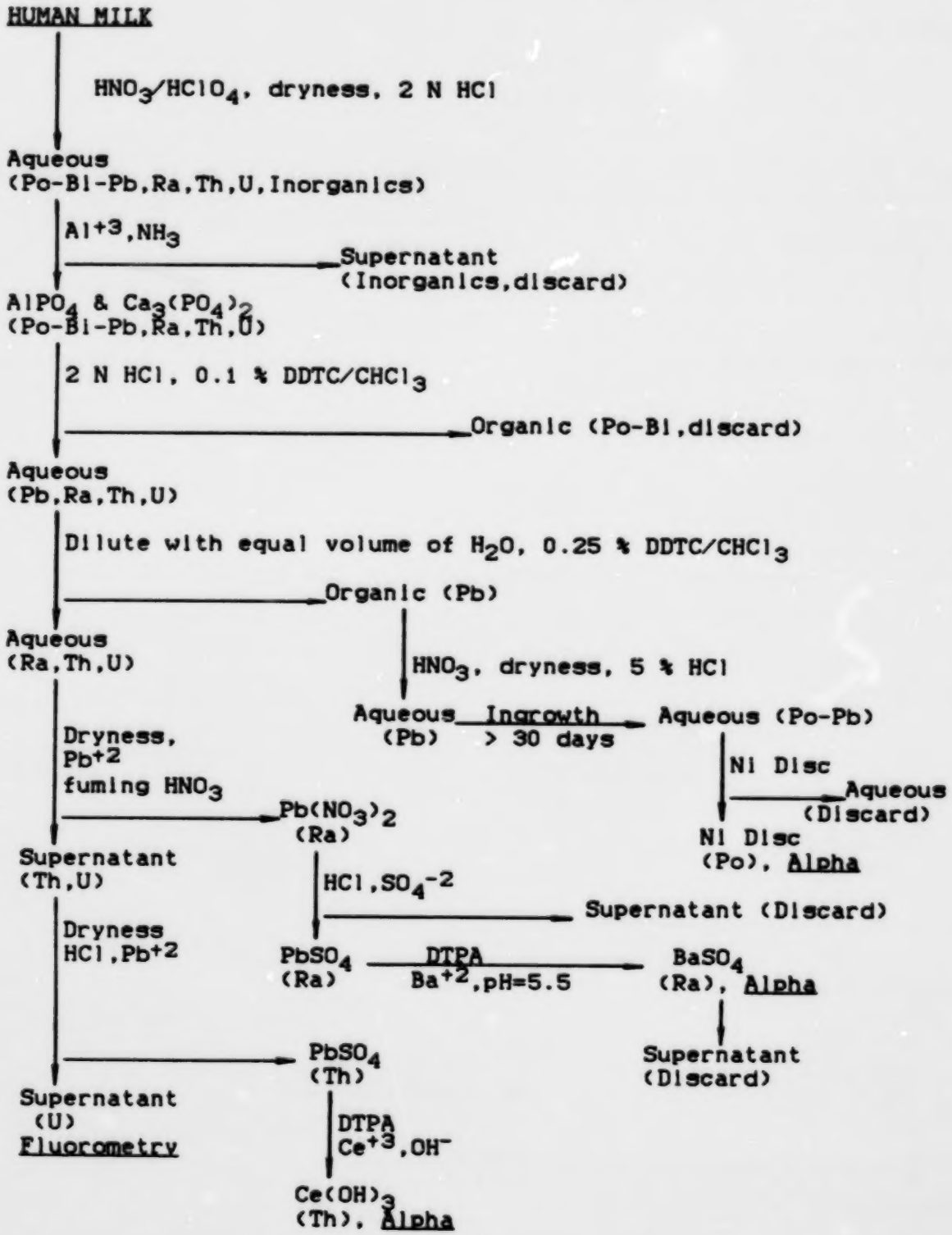

FIGURE 1. Separation Scheme 1.

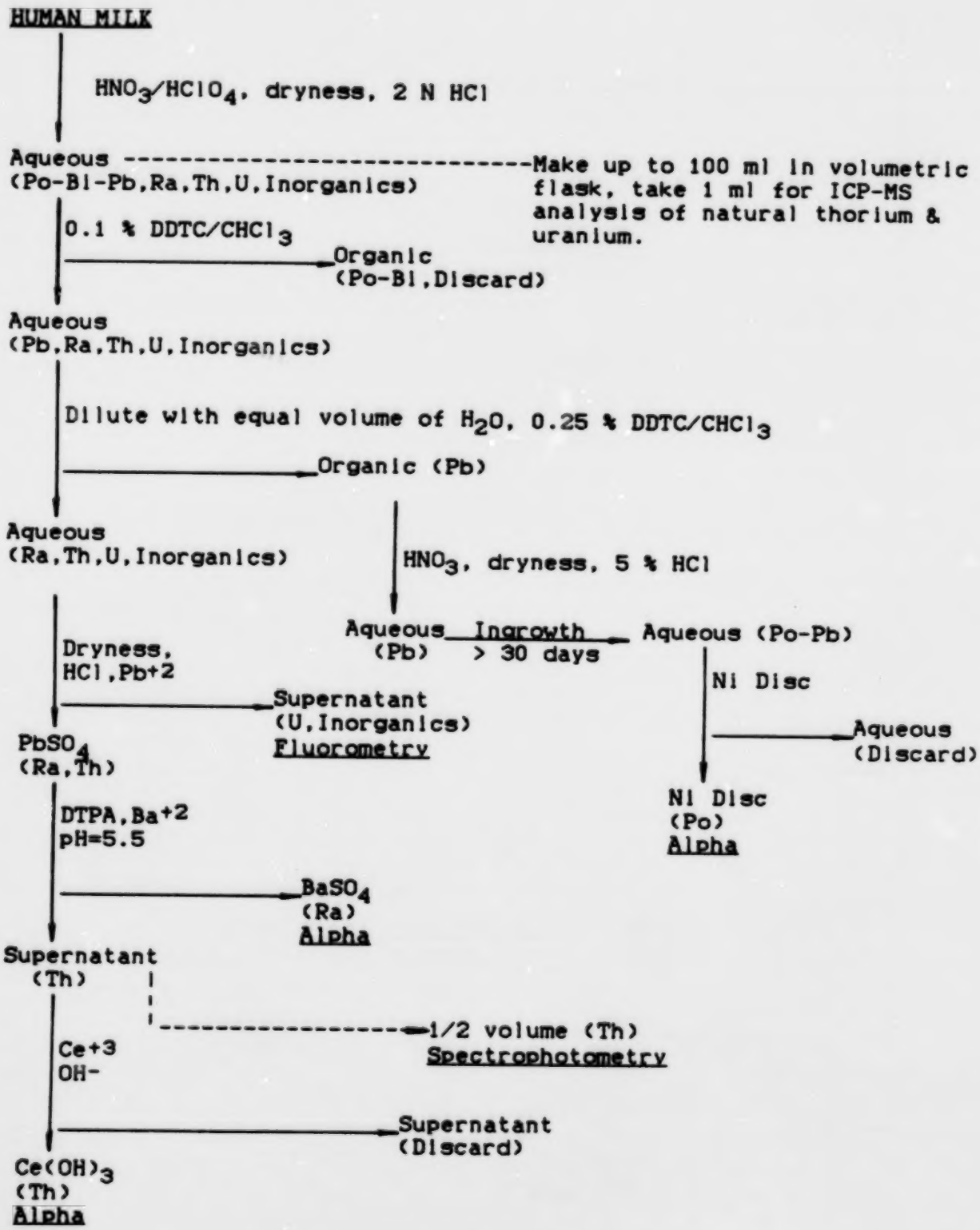

FIGURE 2. Separation Scheme 2.

To obtain Ra-226, one must correct for the presence of Ra-223 and Ra-224 by differential analysis. Radon emanation is an indirect technique, based on the relationship between the activity of the Rn-222 daughter measured to that of the Ra-226 parent present. With this technique, there may be a considerable time delay, since an ingrowth period of twenty days is required to allow the activity of Rn-222 to reach 95% of its parent Ra-226 activity. The gamma spectroscopy technique is an indirect measurement of emitted gamma photons. For the determination of Ra-226, either its photo peak at 185 KeV, or photo peaks of its daughters Pb-214 (295 KeV, 352 KeV) and Bi-214 (609 KeV, 1120 KeV) can be used. Coincidence spectroscopy is based on the separate measurement of the coincident alpha-gamma transitions of Ra-226. High resolution alpha spectroscopy (HRAS) is based on the direct measurement of the alpha emission energy from Ra-226 at 4.8 MeV. The technique simple and specific.

Figure 3a shows an alpha spectrum of Ra-226 upon its separation from other radionuclides. Gradually ingrowth of its daughters Rn-222 (5.5 MeV), Po-218 (6.0 MeV), and Po-214 (7.7 MeV) occur. Their activities reach greater than 98% equilibrium after 25 days as shown in Figure 3b.

Barium-133, a gamma emitter, is used as a tracer for the determination of Ra-226. A known activity of Ba-133 is added to the sample prior to the sample decomposition. The separation of Ra-226 by coprecipatation with barium sulfate allows the calculation of Ba-133 and hence Ra-226 recoveries. Barium-133 is measured by gamma counting.

2.4.2 Thorium Isotopes

The long-lived thorium isotopes, Th-230 from the uranium series, Th-232 and Th-228 from the thorium series, emit alpha particles with energies of 4.6 MeV, 4.0 MeV and 5.3 MeV,

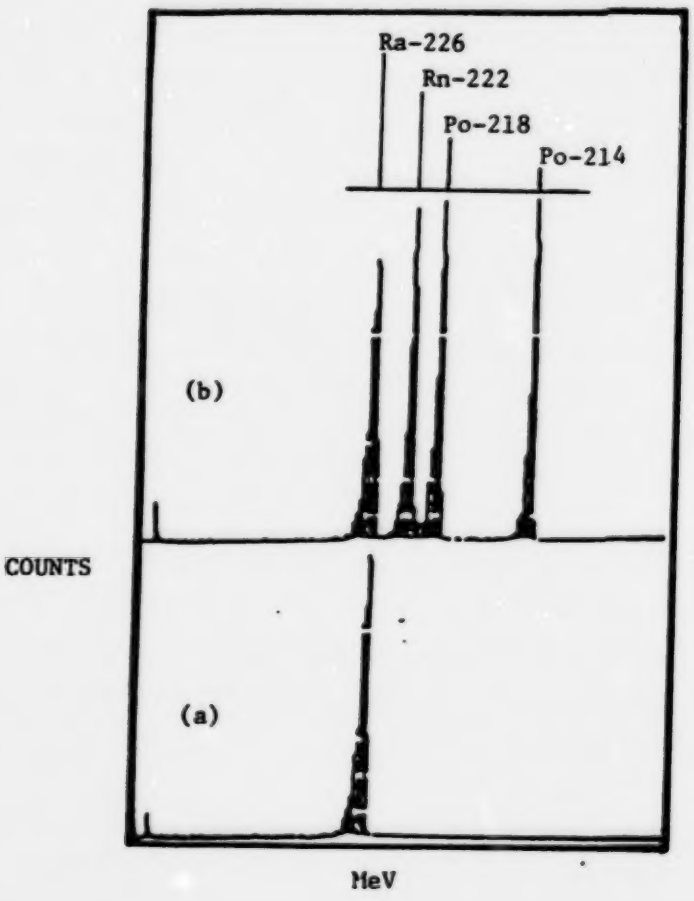

FIGURE 3. Alpha Particle Spectra of Ra-226

(a) Immediately after separation

(b) After 25 days

respectively. They can be determined simultaneously, after separation from all other radionuclides, by high resolution alpha spectroscopy. For best resolution, a uniform and thin source is mandatory. This can be achieved by either electroplating[41] or by coprecipitation with cerous hydroxide[54].

Figure 4a shows an alpha spectrum of the three thorium isotopes upon their separation from other radionuclides. Figure 4b shows four additional peaks at 5.7 MeV, 6.3 MeV, 6.8 MeV and 8.8 MeV due to the ingrowth of Ra-224, Rn-220, Po-216 and Po-212, respectively from Th-228.

Thorium-234, a beta emitter is used as a tracer for the recovery determination of thorium isotopes. A known activity is added to the sample prior to decomposition. The activity recovered is determined by beta counting.

Thorium-232 (natural thorium), due to its low radioactivity per unit mass (0.004 Bq per microgram), can also be determined by spectrophotometry. Several complexing agents can be used including arsenazo III[55-59], and morin[60]. The detection limit is about 0.1 ug/L. At this low level, thorium must be concentrated from a litre of sample for analysis. Recent advances in the inductively coupled argon plasma-mass spectrometry (ICP-MS) make possible the direct determination of thorium at even lower level, 0.01 ug/L, requiring only 10 mL of sample.

2.4.3 Lead-210

Lead-210 emits beta particle having a low emission energy of 0.018 MeV, which is very difficult to measure directly. Therefore, it is usually measured indirectly by alpha spectroscopy of its daughter Po-210 (5.3 MeV, 138 d half-life)[61-71] or by beta measurement of its daughter Bi-210 (1.17 MeV, 5 d half-life)[72-76]. The appropriate technique

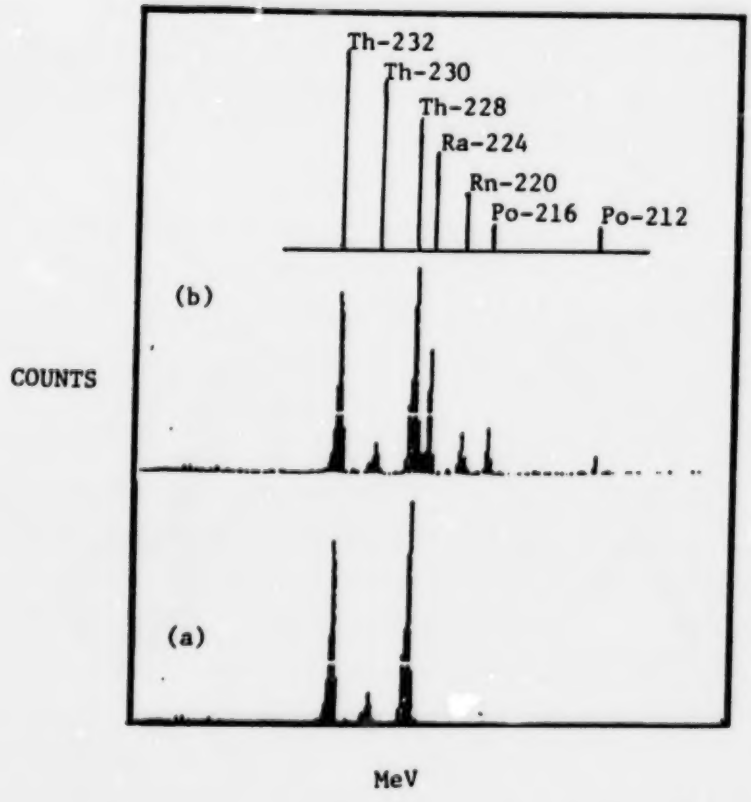

FIGURE 4. Alpha Particle Spectra of Thorium Isotopes

(a) Immediately after separation

(b) After 2 days

depends on the degree of equilibrium between Pb-210, Po-210 and Bi-210.

In the case of human milk samples, the degree of equilibrium is unknown. Two techniques are available. The first is to allow the sample to age for 30 days. At the end of this period, complete equilibrium between Pb-210 and Bi-210 will be ensured to greater than 98%. The Bi-210 is then separated from Pb-210, and its activity determined by beta measurement. The second is to measure Po-210, by high resolution alpha spectroscopy, in a sample one to six months after the removal of the initial Po-210. The activity of Po-210 ingrowth from Pb-210 will be approximately 14%, 26%, 36% and 60% for one, two, three and six months, respectively. An isotopic alpha emitter Po-208 (5.1 MeV) can be used as a tracer for recovery measurement.

Although the radioactivity equilibrium between Pb-210 and Bi-210 is greater than 98% after 30 days, while between Pb-210 and Po-210 is only 14%, the Po-210 technique offers the following advantages. Firstly, it is specific since Po-210 is measured at a discret energy while beta measurement is a gross counting of beta particle emissions having energies from 0.1 MeV to 3.0 MeV. Secondly, there is a potential interference of the beta counting due to Th-234. The latter, a beta emitter, is being used in very high activity as a tracer for the determination of thorium isotopes. Thirdly, high resolution alpha spectroscopy has much lower background and higher sensitivity than beta counting.

Figure 5 shows a typical spectrum of Po-210/Po-208.

2.4.4 Natural Uranium

The analytical chemistry of uranium was extensively investigated as part of the Manhattan project[77]. Since then, a number of measurement techniques have been developed. These

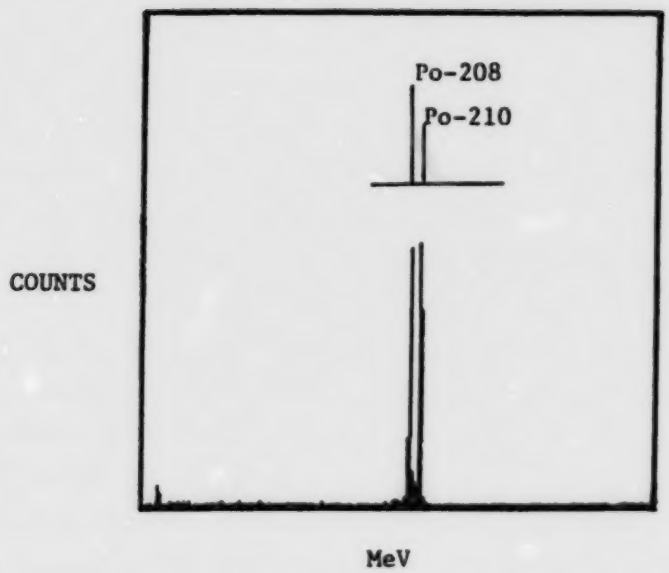

FIGURE 5. Alpha Particle Spectrum of Polonium

include fluorometric, gravimetric, volumetric, spectrophotometric, coulometric, polarographic, alpha spectroscopy, gamma spectroscopy, neutron activation analysis, fission track analysis, and X-ray fluorescence. Several review articles and manuals have been published[77-82].

For routine analysis, fluorometric technique is the most common one. Uranium, when fused with sodium fluoride, produces a brilliant yellow green fluorescence when exposed to ultraviolet light. This technique is specific for uranium when the excitation wavelength is 365 nm. The intensity of the fluorescence is proportional to the amount of uranium present. The detection limit is about 0.5 ug/L. Substantially lower limit at 0.01 ug/L is now possible by using ICP-MS technique.

2.4.5. Activity Levels of Radionuclides

For the purpose of testing both the effectiveness of the separation and determination techniques and the detection limits, known activities of radionuclides were added to the milk samples. Since there is no available information concerning activity levels of radionuclides of the uranium and thorium series in human milk, an arbitrary maximum level I and five fractional levels II, III, IV, V and VI were chosen for testing. Due to the difficulty in securing Th-230 standard, Th-228 was used instead. Table 2 lists the activities of radionuclides chosen.

Since only one milk bank was found which agreed to provide a limited supply of human milk for this project, it was decided that the testing of separation and determination be conducted initially using synthetic solutions. All these solutions contained the same major inorganic constitutents present in human milk with different levels of radionuclides. Each 100 ml aliquot represented the resulting solution from the decomposition of one litre of human milk.

Two litres of composite human milk were supplied by the milk bank. One litre was spiked with level IV radionuclides and the other was not. Three 250 mL aliquots from each of the spiked and unspiked milk were tested. Health and Welfare Canada also supplied one litre of human milk. Three 250 mL aliquots were taken without spike and tested.

TABLE 2. Activity Levels of Radionuclides

RADIONUCLIDE	LEVEL I Bq/L (μg/L)	LEVEL II Bq/L (μg/L)	LEVEL III Bq/L (μg/L)	LEVEL IV Bq/L (μg/L)	LEVEL V Bq/L (μg/L)	LEVEL VI Bq/L (μg/L)
Pb-210	1.40	0.70	0.35	0.17	0.07	0.014
Ra-226	1.00	0.50	0.25	0.10	0.05	0.010
Th-232	0.80 (200)	0.40 (100)	0.20 (50)	0.08 (20)	0.04 (10)	0.008 (2)
Th-228	0.80	0.40	0.20	0.08	0.04	0.008
Uranium	(25)	(12.5)	(6.3)	(2.5)	(1.3)	(0.25)

3. RESULTS AND DISCUSSION

All experimental results using synthetic solutions are tabulated in Tables 3-10 with a summary in Table 11. Table 12 lists results of testing using human milk samples. All results are reported on net basis, i.e., the differences between actual and blank results.

3.1 SEPARATION SCHEMES

Separation Scheme 1 was terminated after only limited testing for two reasons: namely, health hazard due to the use of fuming nitric acid and poor recovery of Ra-226.

Fuming nitric acid is a mixture of nitrogen dioxide in concentrated nitric acid. In an open vessel, the brownish fumes of nitrogen dioxide is easily liberated into the working environment. It is toxic and hence a serious health hazard to the laboratory staff.

The recovery of Ra-226, by means of Ba-133 tracer study was found to be very low, less than 40% (Tables 3 and 4). In this procedure, Ra-226 is separated as radium nitrate by coprecipitation with lead nitrate in 75% nitric acid at $-5^{\circ}C$. The quantity of lead nitrate precipitated varied greatly among replicates. This incomplete precipitation probably caused the poor recovery.

Separation scheme 2 was tested using all six synthetic solutions and human milk samples. The results (Tables 5-12) indicate that this procedure affords good recovery and good reproductibility.

3.2 STATISTICAL TREATMENT OF DATA

The activities of each of five radionuclides, i.e. Th-232, Th-228, Ra-226, Pb-210, and uranium, have been measured in

TABLE 3. Activity Level III (Scheme 1) Results

REPLICATE	Pb-210 0.35 Bq	Ra-226 0.25 Bq
1	100% 0.29±0.04	37% 0.25±0.03
2	90% 0.29±0.04	39% 0.25±0.03
3	96% 0.31±0.04	49% 0.28±0.03
4	78% 0.30±0.04	40% 0.26±0.03
5	95% 0.30±0.04	24% 0.34±0.03
6	92% 0.26±0.04	52% 0.23±0.03
RECOVERY, % MEAN, $\bar{x}$ S.D., σ	92% 0.29 0.02	40% 0.27 0.04

TABLE 4. Activity Level IV (Scheme 1) Results

REPLICATE	Pb-210 0.17 Bq	Ra-226 0.10 Bq
1	91% 0.12±0.02	0.7% 4.6 (?) *
2	98% 0.15±0.02	25% 0.056±0.015 *
3	86% 0.15±0.02	51% 0.108±0.014
4	78% 0.14±0.02	49% 0.093±0.014
5	88% 0.11±0.02	24% 0.107±0.020
6	64% 0.11±0.02	28% 0.114±0.021
RECOVERY, % MEAN, $\bar{x}$ S.D., σ	84% 0.13 0.02	38% 0.106 0.010

* Outlier

TABLE 5. Activity Level I (Scheme 2) Results

REPLICATE	Pb-210 1.40 Bq	Ra-226 1.00 Bq	Th-228 0.80 Bq	Th-232 0.80 Bq	U 25 µg
1	98% 1.29±0.12	73% 0.98±0.10	98% 0.66±0.08	98% 0.67±0.08	25.
2	100% 1.45±0.13	73% 1.14±0.10	100% 0.78±0.09	100% 0.79±0.09	27.
3	100% 1.37±0.13	75% 1.14±0.10	98% 0.73±0.09	98% 0.79±0.09	27.
4	100% 1.41±0.13	78% 1.05±0.10	97% 0.73±0.09	97% 0.76±0.09	23.5
5	86% 1.65±0.14	70% 1.11±0.10	95% 0.77±0.09	95% 0.71±0.09	23.
6	98% 1.49±0.13	75% 1.06±0.10	98% 0.75±0.09	98% 0.80±0.09	25.
RECOVERY, % MEAN, $\bar{x}$ S.D., σ	97% 1.44 0.12	74% 1.08 0.06	98% 0.74 0.04	98% 0.75 0.04	25.1 1.7

TABLE 6. Activity Level II (Scheme 2) Results

REPLICATE	Pb-210 0.70 Bq	Ra-226 0.50 Bq	Th-228 0.50 Bq	Th-232 0.40 Bq	U 12.5 µg
1	100% 0.80±0.08	69% 0.57±0.06	93% 0.33±0.04	93% 0.35±0.04	12.5
2	100% 0.68±0.08	77% 0.48±0.05	100% 0.35±0.04	100% 0.38±0.05	12.
3	92% 0.76±0.08	74% 0.50±0.05	95% 0.33±0.04	95% 0.38±0.05	14.5
4	95% 0.68±0.08	77% 0.46±0.05	96% 0.38±0.05	96% 0.37±0.05	14.5
5	95% 0.64±0.08	74% 0.50±0.05	46%* 0.36±0.04	46%* 0.38±0.05	12.
6	100% 0.52±0.08	79% 0.50±0.05	95% 0.34±0.04	95% 0.38±0.05	12.5
RECOVERY, % MEAN, $\bar{x}$ S.D., σ	97% 0.68 0.10	75% 0.50 0.04	96% 0.35 0.02	96% 0.37 0.01	13. 1.2

* Approximately one-half of solution spilled.

TABLE 7. Activity Level III (Scheme 2) Results

REPLICATE	Pb-210 0.35 Bq	Ra-226 0.25 Bq	Th-228 0.20 Bq	Th-232 0.20 Bq	U 6.2 μg
1	96% 0.31±0.05	78% 0.24±0.03	92% 0.15±0.02	92% 0.18±0.02	4.8
2	95% 0.34±0.05	60% 0.27±0.03	98% 0.21±0.03	98% 0.20±0.03	6.0
3	97% 0.22±0.04	81% 0.27±0.03	98% 0.20±0.03	98% 0.19±0.02	5.0
4	98% 0.37±0.06	84% 0.26±0.03	94% 0.23±0.03	94% 0.19±0.02	5.0
5	94% 0.28±0.04	83% 0.26±0.03	94% 0.18±0.02	94% 0.19±0.02	5.0
6	100% 0.24±0.04	83% 0.30±0.03	94% 0.20±0.03	94% 0.20±0.03	5.5
RECOVERY, % MEAN, $\bar{x}$ S.D., σ	97% 0.29 0.06	78% 0.27 0.02	95% 0.20 0.03	95% 0.19 0.01	5.2 0.5

TABLE 8. Activity Level IV (Scheme 2) Results

REPLICATE	Pb-210 0.17 Bq	Ra-226 0.10 Bq	Th-228 0.08 Bq	Th-232 0.08 Bq	U 2.5 µg
1	88% 0.16±0.03	58% 0.112±0.013	95% 0.076±0.009	95% 0.089±0.011	1.5
2	100% 0.10±0.03	65% 0.116±0.014	91% 0.068±0.008	91% 0.074±0.009	2.3
3	100% 0.21±0.04	59% 0.095±0.010	93% 0.079±0.009	93% 0.078±0.009	2.6
4	89% 0.15±0.03	60% 0.096±0.010	99% 0.092±0.011	99% 0.077±0.009	1.5
5	86% 0.16±0.03	57% 0.091±0.009	95% 0.078±0.008	95% 0.088±0.011	1.5
6	100% 0.12±0.03	56% 0.118±0.012	96% 0.082±0.010	96% 0.081±0.010	2.0
RECOVERY, % MEAN, $\bar{x}$ S.D., σ	94% 0.15 0.04	59% 0.105 0.012	95% 0.079 0.008	95% 0.081 0.006	1.9 0.5

TABLE 9. Activity Level V (Scheme 2) Results

REPLICATE	Pb-210 0.07 Bq	Ra-226 0.05 Bq	Th-228 0.04 Bq	Th-232 0.04 Bq	U 1.2 µg
1	85% 0.069±0.012	78% 0.060±0.009	91% 0.044±0.005	91% 0.041±0.005	1.4
2	89% 0.046±0.009	73% 0.060±0.009	90% 0.044±0.005	90% 0.043±0.005	1.2
3	88% 0.046±0.009	67% 0.062±0.009	89% 0.031±0.004	89% 0.035±0.004	0.6
4	94% 0.051±0.010	58% 0.064±0.011	90% 0.046±0.006	90% 0.037±0.004	0.9
5	92% 0.054±0.010	72% 0.041±0.005	93% 0.044±0.005	93% 0.043±0.005	1.1
6	90% 0.064±0.010	67% 0.039±0.005	96% 0.042±0.005	96% 0.037±0.004	0.8
RECOVERY, % MEAN, $\bar{x}$ S.D., σ	90% 0.055 0.010	69% 0.052 0.011	92% 0.042 0.005	92% 0.039 0.003	1.0 0.3

TABLE 10. Activity Level VI (Scheme 2) Results

REPLICATE	Pb-210 0.014 Bq	Ra-226 0.010 Bq	Th-228 0.008 Bq	Th-232 0.008 Bq	U 0.25 µg
1	88% 0.013±0.005	68% 0.010±0.003	91% 0.012±0.003	91% 0.009±0.002	< 0.15
2	90% 0.015±0.005	68% 0.016±0.004	87% 0.012±0.003	87% 0.009±0.002	< 0.15
3	92% 0.008±0.005	79% 0.008±0.002	93% 0.011±0.003	93% 0.008±0.002	< 0.15
4	89% 0.008±0.005	67% 0.009±0.003	91% 0.013±0.004	91% 0.007±0.002	< 0.15
5	90% 0.008±0.005	78% 0.010±0.003	87% 0.009±0.002	87% 0.008±0.002	< 0.15
6	86% 0.015±0.005	65% 0.011±0.003	88% 0.011±0.003	88% 0.009±0.002	< 0.15
RECOVERY, % MEAN, $\bar{x}$ S.D., σ	89% 0.011 0.004	71% 0.011 0.003	90% 0.011 0.001	90% 0.008 0.001	< 0.15

TABLE 11. Summary of Results

ACTIVITY		TABLE I	TABLE II	TABLE III	TABLE IV	TABLE V	TABLE VI
Ra-226	A	1.00	0.50	0.25	0.10	0.05	0.01
	M-1			0.27±0.04	0.106±0.010		
	M-2	1.08±0.06	0.50±0.04	0.27±0.02	0.105±0.012	0.052±0.011	0.011±0.003
Th-228	A	0.80	0.40	0.20	0.08	0.04	0.008
	M-2	0.74±0.04	0.35±0.02	0.20±0.03	0.079±0.008	0.042±0.005	0.011±0.001
Th-232	A	0.80	0.40	0.20	0.08	0.04	0.008
	M-2	0.75±0.04	0.37±0.01	0.19±0.01	0.081±0.006	0.039±0.003	0.008±0.001
Pb-210	A	1.40	0.70	0.35	0.17	0.07	0.014
	M-1			0.29±0.02	0.13±0.02		
	M-2	1.44±0.12	0.68±0.10	0.29±0.06	0.15±0.04	0.055±0.010	0.011±0.004
Uranium	A	25.	12.5	6.3	2.5	1.3	0.25
	M-2	25.1±1.7	13.0±1.2	5.2 ±0.5	1.9 0.5	1.0±0.3	< 0.15

NOTES: A - Added Activity
M-1 - Measured Activity (Scheme 1)
M-2 - Measured Activity (Scheme 2)

TABLE 12. Human Milk Results (Scheme 2)

SAMPLE	Po-210 0.043 Bq*	Pb-210 0.043 Bq*	Ra-226 0.025 Bq*	Th-228 0.020 Bq*	Th-232		U 0.62 µg*
					0.020 Bq*	5 µg*	
1*	0.042±0.006	0.041±0.008	0.029±0.006	0.018±0.004	0.020±0.004	4.3	0.40
2*	0.054±0.006	0.045±0.008	0.022±0.004	0.024±0.004	0.015±0.004	5.1	0.45
3*	0.047±0.006	0.033±0.008	0.022±0.004	0.018±0.004	0.016±0.004	5.3	0.50
MEAN, $\bar{x}$	0.048	0.040	0.024	0.020	0.017	4.9	0.45
S.D., σ	0.006	0.006	0.004	0.003	0.003	0.5	0.05
4	< 0.005	< 0.010	< 0.005	< 0.005	< 0.005	0.06	< 0.02
5	< 0.005	< 0.010	< 0.005	< 0.005	< 0.005	< 0.02	< 0.02
6	< 0.005	< 0.010	< 0.005	< 0.005	< 0.005	0.02	< 0.02
7	< 0.005	< 0.010	< 0.005	< 0.005	< 0.005	< 0.02	< 0.02
8	< 0.005	< 0.010	< 0.005	< 0.005	< 0.005	< 0.02	< 0.02
9	< 0.005	< 0.010	< 0.005	< 0.005	< 0.005	< 0.02	< 0.02
10	< 0.005	< 0.010	< 0.005	< 0.005	< 0.005	< 0.02	< 0.02

NOTE: Samples 1 to 6, 250 ml aliquots of human milk from Medicine Hat Hospital
Samples 7 to 9, 250 ml aliquots of human milk from Health & Welfare Canada
Sample 10, reagent blank

* Activities of radionuclides added to Samples 1 to 3 prior to digestion

synthetic milk solution at six activity concentrations. For each data point (i.e. a given concentration of a given radionuclide) six replicate analyses have been performed. Thus, the best estimates of the mean, $\bar{x}$, and standard deviation, σ, for each point may be obtained from:

$$\bar{x} = \frac{1}{n}\sum x_i$$

$$\sigma = \left[\sum (x_i - \bar{x})^2 / (n - 1)\right]^{0.5}$$

where n in this case is 6. For each radionuclide, a seventh data point has been provided by using aliquots of human milk to which a low activity level has been added. Three rather than six replicate analyses were made, and means and standard deviations calculated in the manner outlined above.

In order to investigate the relationship between measured y, and added activities x, we have analysed the correlation between them, assuming a linear relation between the two. Thus, obviously if x and y are equal for all data points, a line

$$y = x$$

will be a perfect fit. In general we expect the data to be scattered about a line

$$y = a + bx$$

A significantly high value of the intercept, a, will indicate that a measurable amount of activity might be found in essentially "blank" solutions.

Deviations in the value of the slope, b, from unity would introduce systematic errors in the accuracy of the measured activity. (In this respect, the accuracy of the added activity is assumed to be better than the precision to which it can be diluted and dispensed.)

The derivation of the best fit straight line has been undertaken using the so-called "method of effective variance" described by Lybanon[83]. In this method, each data point is weighted by the vector addition of its x and y variances. The best fit straight line is that for which the sum of squares of distances perpendicular to the line, is minimized.

A computer programme[83] has been used to generate the best fit straight line of measured activity versus added activity (Figures 6-10). It also provides values of the derived intercept, a, slope, b, along with estimates for the standard deviation of each quantity and Chi squared (Table 13).

For example, the output provided by the programme using the data for Th-232 activity can be examined.

The fit parameters found are (rounded off to two significant figures)

$$a = 7.6 \times 10^{-4} \pm 5.2 \times 10^{-4}$$

$$b = 0.94 \pm 0.02$$

Chi squared is equal to 1.33 for 5 degrees of freedom.

We conclude that the 7 data points (6 derived from synthetic and 1 from human milk) are well fitted by a straight line. The value of Chi squared is significant at about the 94% level,

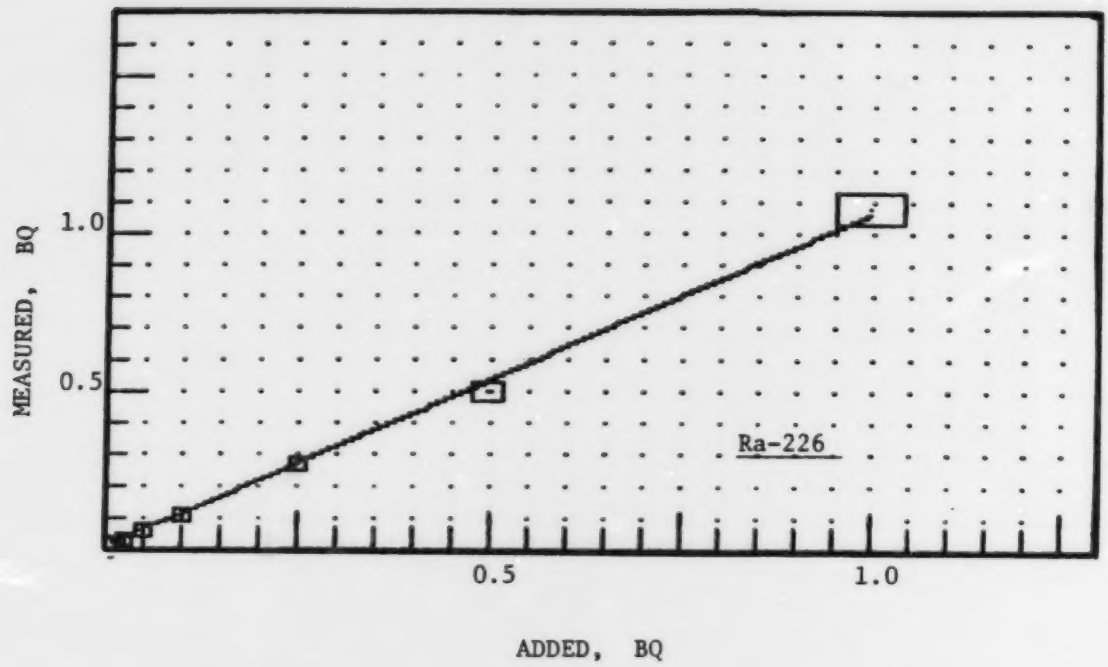

FIGURE 6. Plot of Measured vs. Added Ra-226

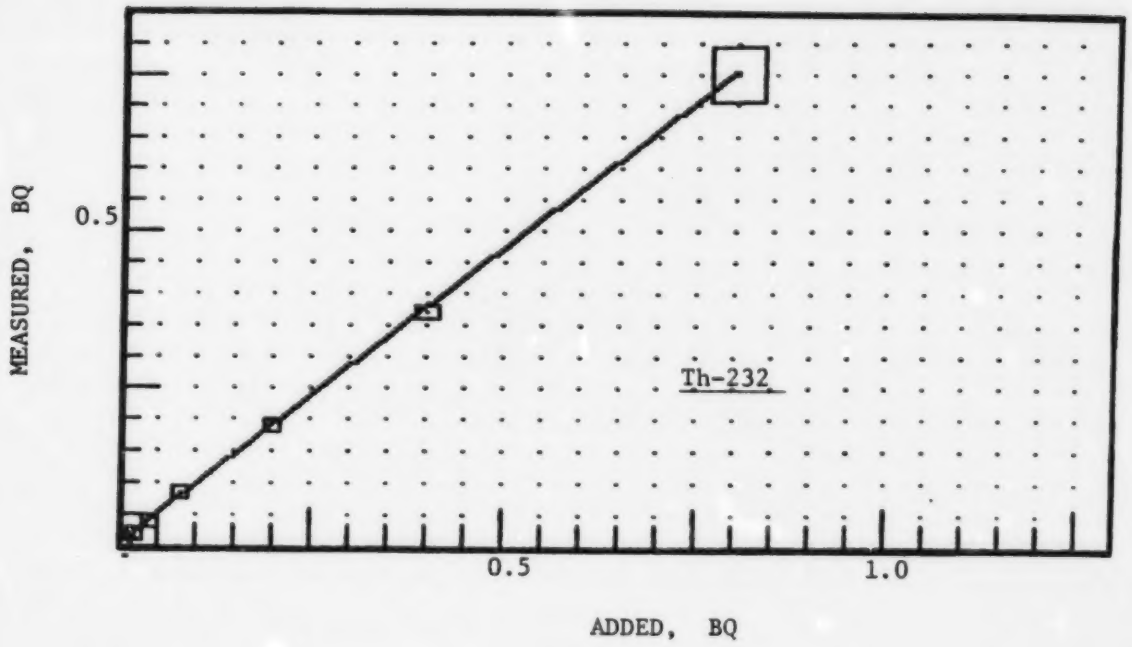

FIGURE 7. Plot of Measured vs. Added Th-232

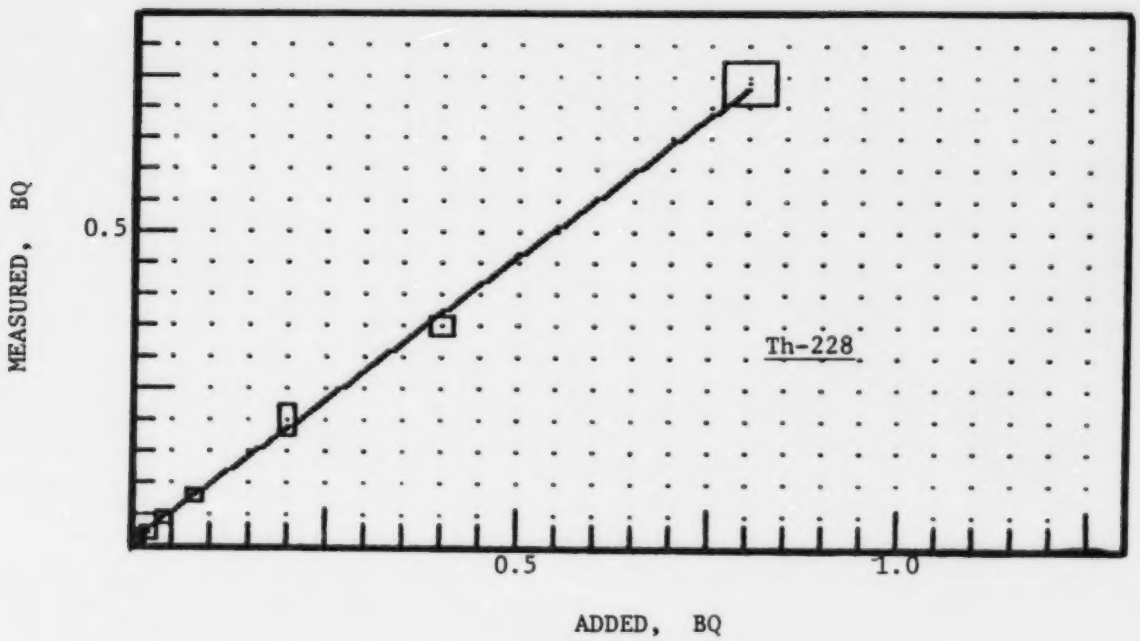

FIGURE 8. Plot of Measured vs. Added Th-228

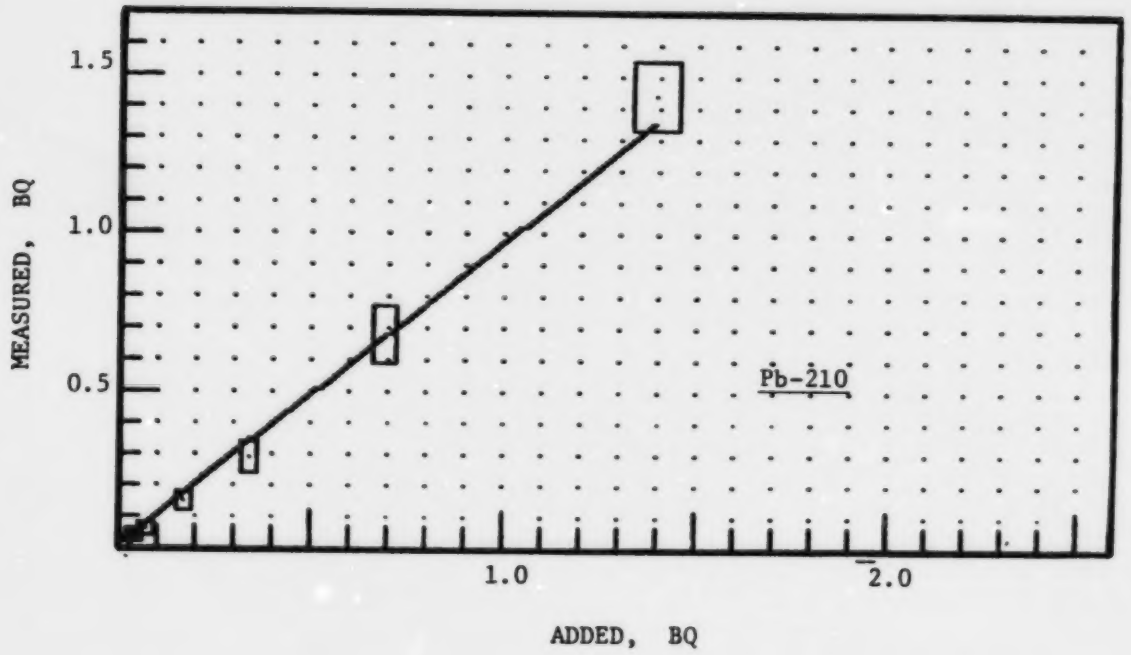

FIGURE 9. Plot of Measured vs. Added Pb-210

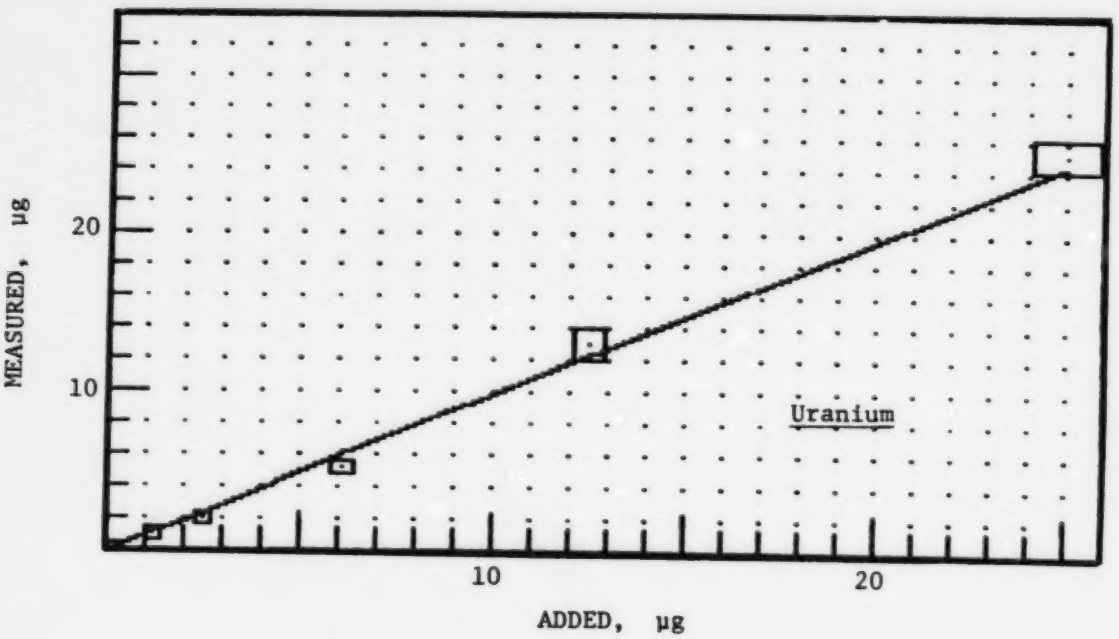

FIGURE 10. Plot of Measured vs. Added Uranium

TABLE 13. Best Fit Parameters

ISOTOPE	Intercept, a	Slope, b	Chi Square	Degree of Freedom
Ra-226	-0.0005 ± 0.001	1.06 ± 0.02	0.77	5
Th-232	0.0008 ± 0.0005	0.94 ± 0.02	1.33	5
Th-228	0.004 ± 0.0007	0.90 ± 0.02	1.31	5
Pb-210	-0.003 ± 0.002	0.96 ± 0.04	1.92	5
Uranium	-0.27 ± 0.11	0.98 ± 0.04	2.30	4

i.e. there is a risk of about 6% that the correlation is due to chance. The more commonly used correlation coefficient, r, cannot be applied to the effective variance fitting technique, but it is interesting to note that a straightforward unweighted fitting procedure would yield, r, in excess of 0.99 which is significant at, at least the 98% level.

The fit parameters indicate that we might obtain a measured activity of

$$7.6 \pm 5.2 \times 10^{-4} \text{ Bq}$$

for a blank sample. Even at the two standard deviation level this amounts to only about 0.002 Bq or a quarter of the smallest activity measured (0.008 Bq).

The other fit parameters, 0.94 ± 0.02 shows that measured quantities might on average be about 6% below the added activities. Such a systematic error can, of course, be corrected for provided that we can tolerate a standard deviation of 2%.

Data analyses for the other radionuclides allow similar conclusions to be drawn.

The analysis for Ra-226 activities shows the least amount of scatter (Chi squared is only 0.77 for five degrees of freedom). The slope is 1.05 ± 0.02 showing that measured quantities might be 5% higher with a standard deviation of 2%. It may be interesting to note that while radium data seem to be statistically good, some recovery values listed are not so high as for thorium. We concude that the chemical difficulties encountered in isolating radium are offset by the higher specific activity thus providing more active alpha particle sources per unit weight.

The straight line fit parameters for the determination of uranium by fluorometry are:

$$a = -0.27 \pm 0.11$$

$$b = 0.98 \pm 0.04$$

We conclude that atomic fluorescence measurements are insensitive to very low concentrations, i.e. that there is essentially no measureable signal until the added activity is increased to about 0.3 microgram (the intercept of the line on the x axis). The scatter about the fitted line yields a Chi squared value of 2.3 at 4 degrees of freedom.
There are not enough mass spectrometric results (Table 12) to carry out a statistical analysis involving straight line fitting. It appears that mass spectrometry (ICP-MS) is more sensitive than alpha spectrometry and fluorometry for thorium and uranium determinations, respectively. The achievable accuracy needs to be investigated further using more replicable measurements.

The inclusion of actual milk sample data along with synthetic within the same set does not reveal any inconsistency. If a small amount of intrinsic activity were present in the natural samples, we might expect that the measured activity value would be shifted to a position above the line fitted to the entire data set. Measurements made on blank samples yielded the same detector count rate as that which occurs with no sample inserted into the counting chamber. The measured upper limit on the activity is obtained for the usual 2-hour counting time allocated to samples. A more realistic limiting activity is derived from the intercept of the straight line fit to the data as outlined above since the intercept is based on about 40 measurements at various concentrations.

3.3 CONCLUSIONS

Trials of the radiometric procedure reveal that the measured activities of Th-232, Th-228, Ra-226, Pb-210, and uranium are in close (to within a few percent) agreement with the activities added to both synthetic and natural milk. The best fit straight line through the data points on the "measured versus added" plot passes close to the origin in all cases. A small intercept implies some difficulty in distinguishing whether activity is present if the detection level is set as low as tenths of Bq or even lower. We would expect counting statistics to be a limiting factor at such levels. Considering 0.1 Bq for example, at a geometry factor of 0.2 for an alpha detector, we would expect a count rate of only about 1.4 counts per minute. Even with no corrections for background, over 4 hours of counting would be required to reduce the Poisson error to $\pm$5%. Likewise, for 0.05 Bq, 0.025 Bq, 0.010 Bq, and 0.005 Bq, the Poisson errors are $\pm$8%, $\pm$11%, $\pm$17% and $\pm$25%, respectively. If a measured activity with $\pm$10% error is acceptable, then the detection limit should be set at 0.025 Bq/L. However, if only a fraction of a litre of sample is available, the level must be increased. For example, the detection limit for 250 mL sample should be increased to 0.1 Bq/L.

Replicate measurements would improve matters somewhat. We conclude that if the means of Ra-226 activity in milk from two locations went from 0.05 Bq to 0.1 Bq, six replicable measurements at each location would yield a student "t" value given by:

$$t = (\bar{x}_1 - \bar{x}_2) / \left[(\sigma_1^2 / n_1) + (\sigma_2^2 / n_2) \right]^{0.5}$$

of 7.5 which is highly significant.

Similar calculations can be performed to determine how many samples are required to establish significant evidence for action to be taken at a given location.

Improved counting statistics could be obtained by allocating counting equipment for more time to fewer samples. However, there is no substitute for numerous replicate measurements if the true variance of the parent population is to be estimated properly. The variance of the mean will decrease by the reciprocal of the number of replicate measurements, and thus the degree of confidence will increase as to whether the mean exceeds or is less than some established regulatory level.

The accuracy of all determined values are traceable to either pure chemical compounds (certified reagents such as uranium or thorium salts) or to radiometric standards certified by national laboratories (e.g. NBS, NPL etc). It would appear that precision rather than accuracy may be the limiting factor in these determinations.

The methodology developed in this study is recommended. The reasons are as follows:

(1) The nitric acid and perchloric acid decomposition technique offers complete destruction of all inorganic matter present in human milk samples. In addition, no foreign cations or impurities, other than hydrogen, are introduced, which might complicate the separation and determination of radionuclides.

(2) The sequential separation scheme 2 (Figure 2), offers good recovery of all radionuclides of interest. These include Pb-210, Ra-226, thorium isotopes (Th-228, Th-230 and Th-232), and natural uranium.

(3) The complete method offers accurate and precise measurements.

4. RECOMMENDED METHODOLOGY

4.1 FIELD SAMPLING

4.1.1 Procedure for Sampling

(1) Wash hands well and cleanse nipple with water just prior to expression. Soap tends to dry the nipple and may lead to soreness. If breast pump is to be used, all parts of the pump must be washed with hot, soapy water, rinsed well and boiled in a covered pan for five minutes. Pour off water and keep in the covered pan until ready to use.

(2) Take a plastic collection bottle supplied, fill in the information requested on the attached label.

(3) Remove screw cap, express milk directly into the bottle.

(4) Replace cap on the bottle, close securely and place in refrigerater immediately.

(5) Repeat expression of milk into the same collection bottle for the next 24 - 48 hours. Returning bottle to the refrigerator after each expression.

(6) When the total volume, approximately 250ml, is reached (minimum of 200 ml) store the bottle in the refrigerator freezer compartment or preferrably in a deep freezer, if available, until collection by a local program representative.

4.1.2 Procedure for Shipping

Important: Local program representative should pre-arrange with courier for pick-up time. All samples should be kept frozen as long as possible prior to shipping, especially during

the summer. No shipment is to be made if the expected arrival date at laboratory is on a weekend or holiday, as delivery will not be made. As soon as shipment has been picked up, inform the laboratory, by telephone, the expected arrival time, name of courier and bill of lading number.

(1) Collect frozen samples from donors. Check that all information requested on the attached label is complete and the collection bottle is securely closed.

(2) Place each bottle in a plastic bag and tie with a twist tie.

(3) Place bottles in foam cooler vertically, never on their sides. Add frozen gel freezer packs, ice cubes, or dry ice.

(4) Replace foam cooler cover and tape securely using plastic packaging tape.

(5) Attach address label and warning labels supplied. Such as "This Side Up", "Fragile", "Perishable", and "Keep Cool".

(6) Keep in freezer if possible and wait for pick-up by courier.

4.2 LABORATORY PROCEDURES

4.2.1 Precaution in the use of Perchloric Acid

Because of the potential explosive reaction between perchloric acid and organic matter, all persons should be thoroughly familiar with its hazards and handling techniques [22-24] prior to using this acid. Some safety practices follow:

(1) All work must be done in a fumehood designed for perchloric acid.

(2) Use gogles, barrier shields other devices as necessary for personal protection.

(3) Never add concentrated (>60%) perchloric acid to the sample.

(4) Never use organic chemicals/solvents in the perchloric acid fumehood.

(5) Remove spilled acid by immediately and thoroughly washing with large amounts of water.

4.2.2 Apparatus

Perchloric acid fumehood: Constructed of #316 stainless steel with wash-down capabilities to clean the blower, exhaust ducting and interior walls, ceiling, and working surface.

Fumehood: Regular.

Alpha spectrometer: Surface barrier or silicon diffused junction detector, preamplified, bias voltage supply, linear amplifier, and multichannel analyzer.

Beta counting system: Low background

Gamma Counting system: NaI crystal, power supply, pulse height analyzer.

Centrifuge

Hotplate/magnetic stirrer: Six-place with single speed controller and single heat controller.

Magnetic stirrer: Six-place with single speed controller.

Hot water bath

Vortex mixer

Hotplate

Polonium deposition apparatus: See Figure 11

Filter holder: Conical, for 25 mm membrane filter with stainless steel support screen.

Membrane filters: Cellulose acetate and PVC or PTFE, 25-mm diameter, 0.2-micron pores.

Nickel discs: 25-mm diameter X 0.25-mm.

Plastic discs: 25-mm diameter X 2-mm.

Centrifuge tubes: Conical, 40-ml capacity.

Miscellaneous glasswares and plasticwares

4.2.3 Reagents

Nitric acid (16 N): Reagent grade

Perchloric acid (60-70%): Reagent grade

Hydrochloric acid (12 N): Reagent grade

Perchloric/Nitric mixture (1:1): Mix equal volumes of 16 N nitric acid and 60 - 70% perchloric acid.

Hydrochloric acid (2 N): Dilute 175 mL of 12 N hydrochloric acid with deionized water to 1 L.

Hydrochloric acid (5% and 10%): Dilute 50 ml and 100 ml of 12 N hydrochloric acid to 1 L with deionized water.

DDTC in chloroform (0.1% and 0.25%): Dissolve 0.1 g and 0.25 g of diethylammonium diethyldithiocarbamate in 100 mL of chloroform. Prepare daily.

Potassium sulfate: Reagent grade.

Lead nitrate solution (32%): Dissolve 32 g of lead nitrate in deionized water and dilute to 1 L.

Alkaline DTPA (0.2 M): Dissolve 38 g of sodium hydroide in 800 mL of deionized water and add 80 g of diethylenetriaminepenta-acetic acid while stirring. Cool, adjust pH to 10.6 with 10 N NaOH or 10% HCl and dilute to 1 L.

Sodium hydroxide solution (10 N): Dissolve 40 g of NaOH in deionized water and dilute to 100 mL.

Sodium sulfate/acetic acid solution: Dissolve 400 g of anhydrous sodium sulfate in deionized water, add 80 mL of glacial acetic acid and dilute to 1 L.

Barium carrier solution (1 mg Ba per mL): Dissolve 180 mg of barium chloride dihydrate in deionized water and dilute to 100 mL.

Cerium carrier solution (0.5 mg Ce per mL): Dissolve 105 mg of cerons nitrate hexahydrate in 100 mL of 0.1 N nitric acid solution. Following steps 1 to 7 in Section 2.7 below, prepare

a blank cerium hydroxide source. Determine the alpha particle spectrum. If Th-232 and its daughters are present, purify the carrier according to the published procedure[33].

Alcohol (80%): Mix 4 volumes of denatured alcohol with 1 volume of deionized water.

Lead-210 standard solution (1 Bq/mL): Dilute NBS traceable Pb-210 in 0.1 N HCl.

Radium-226 standard solution (1 Bq/mL): Dilute NBS traceable Ra-226 in 0.1 N HCl.

Thorium-228 standard solution (1 Bq/mL): Dilute NBS traceable Th-228 in 0.1 N HCl.

Natural thorium standard solution (100 mg/L): Dissolve 207 mg of pure thorium nitrate in 0.1 N HCl and dilute to 1 L.

Natural uranium standard solution (100 mg/L): Dissolve 117.9 mg of pure uranium oxide (U_3O_8) in 20 mL of 16 N HNO_3 with heating. Cool and dilute to 1 L with deionized water.

Tracers: Ba-133, Th-234, and Po-208.

4.2.4 Decomposition of Human Milk Samples

(1) Thaw frozen milk sample to room temperature. Invert bottle to mix.

(2) Measure 250 mL of milk and place it in a 600-mL beaker.

(3) Add 100 mL of 16 N nitric acid and appropriate activities of tracers. Mix thoroughly with a stirring rod.

(4) Boil vigorously on a hotplate set at 200°C until volume is reduced to 100 mL. It takes approximately 4 to 6 hours during which time both evaporation of water and oxidation of organic matter occur. A lower brownish nitric acid layer and a upper yellow fatty layer appear.

(5) Add 50 mL of 16 N nitric acid, cover beaker with watch glass.

(6) Reduce hotplate temperature to 160°C and continue heating until the upper fatty layer disappears and the solution is light brown. It takes 30-40 hours and the volume should be about 50 mL.

(7) Remove beaker from hotplate and allow to cool to room temperature.

(8) Add 100 mL 1:1 mixture of nitric acid and perchloric acid to the beaker. Cover with watch glass.

(9) Return beaker to hotplate, increase its temperature to 175°C and maintain for 8 hours.

(10) Increase hotplate temperature to 200°C and heat for 8-20 hours until the solution is colorless. During this period, constant monitoring is mandatory. As nitric acid slowly evaporates, the concentration of perchloric acid gradually increases. The more resilient organic matter is being oxidized. When oxidation occurs rapidly, free carbon will form. When the solution turns to grey/black color, immediately remove the beaker from the hotplate. Add 1-2 mL 16 N of nitric acid and when the grey/black color disappears, return the beaker to the hotplate. Otherwise, the solution will turn black quickly and explosion is a distinct possibility.

(11) Remove watch glass, allow perchloric acid to evaporate just to dryness. The ash should be white. Do not allow the ash to be baked unneccessarily.

(12) Dissolve residue with 50 mL of hot 2 N hydrochloric acid. Transfer quantitatively to a 100-mL volumetric flask. Rinse beaker twice with 20 mL portions of hot 2 N hydrochloric acid and add to the volumetric flask. Allow to cool to room temperature and make up to volume with 2 N hydrochloric acid. Proceed to Section 4.2.5. for the sequential separation and determination of radionuclides.

4.2.5 Separation and Determination of Radionuclides

4.2.5.1 Lead-210

(1) With a pipetter, transfer exactly 1 mL of the decomposed milk sample solution (Section 4.2.4, step 12) to a 25-mL volumetric flask, make up to volume with deionized water and SAVE for the determination of natural thorium and uranium by ICP-MS (Section 4.2.5.5) . Empty the remaining decomposed milk solution to a 250-mL Erlenmeyer flask, add 15 mL of 0.1% DDTC in chloroform and a magnetic stirring bar. Cover beaker with a watch glass.

WARNING: The handling and evaporation of nitric acid, steps 1 to 8, should be conducted in a well ventilated fumehood. However, DO NOT USE PERCHLORIC ACID FUMEHOOD for this purpose.

(2) Place flask and content in a magnetic stirrer, stir vigorously for 1 minute and allow 3-5 minutes for phase separation.

(3) Using a disposable polyethylene pipette, transfer the DDTC/chloroform extract to a bottle labelled chloroform waste.

(4) Repeat extraction, steps 1 to 3, once.

(5) Dilute the aqueous solution with an equal volume of deionized water. Extract it twice with 15 mL each of 0.25% DDTC in chloroform and transfer the DDTC/chloroform extracts to a 100-mL beaker. RECORD the time as the beginning of Po-210 ingrowth and SAVE the aqueous solution for the separation of Ra-226, thoriums and uranium (Section 4.2.5.2, 4.2.5.3 and 4.2.5.4).

(6) Evaporate the combined extract to dryness on a hotplate set at 60-65°C.

(7) Add 5 mL of 16 N nitric acid to the residue in the beaker, cover with a watch glass. Heat on a hotplate, set at 100°C, until the solution is colorless.

(8) Remove watch glass and evaporate to dryness.

(9) Add 50 mL of hot 5% HCl solution to the beaker, cover with a watch glass and allow to cool to room temperature. Transfer contents to a 125-mL polyethylene bottle.

(10) Repeat step 9 once.

(11) Add a known activity of Po-208 tracer to the bottle, cap securely, invert twice to mix contents and store for 30 days or more to allow the ingrowth of Po-210 from Pb-210.

(12) At the end of the storage period, empty contents to a 250-mL beaker for Po self decomposition on a nickel disc.

(13) Clean nickel disc with chloroform, soak in 12 N HCl for 5 minutes and rinse with deionized water.

(14) Assemble the nickel disc in the holder and place holder in solution as shown in Figure 11.

(15) Using a hotplate/magnetic stirrer, stir the solution moderately and heat the solution to 60-65°C. Allow 2 hours for the decomposition.

(16) Remove nickel disc from holder. Rinse disc with deionized water, then with 80% alcohol and allow to air dry.

(17) Prepare a calibration solution containing an exact activity, A_s, of a standard Po-210 and identical activity of Po-208 tracer, as used in step 11 above, in 100 mL of 5% HCl solution. Also prepare a blank reagent solution.

(18) Deposit both calibrations and blank solutions on nickel discs as in steps 13 to 16.

(19) Count blank, calibration standard and samples in the alpha spectrometer for Po-210 and Po-208. Calculate count rate by dividing the number of counts by length of time counted.

(20) CALCULATIONS

(a) Calculate the apparent calibration constant, F, from the equation

$$F = (r - b) / A_s$$

(b) Calculate the relative chemical yeld factor, Y, the recovery of the sample disc relative to the recovery of the calibration disc, from the equation:

$$Y = (R_t - b_t) / (r_t - b_t)$$

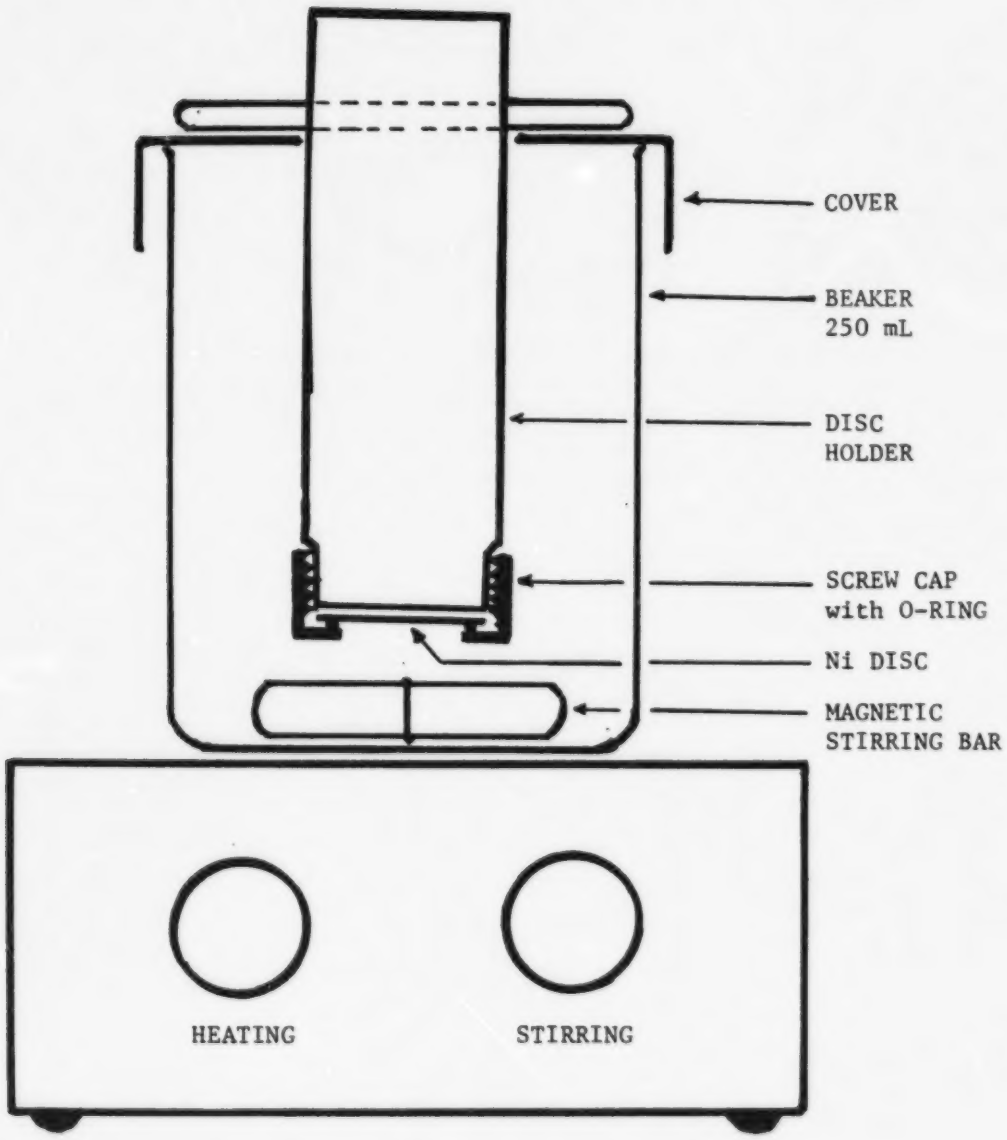

FIGURE 11. Polonium Deposition Apparatus

(c) Calculate the Po-210 alpha activity of the sample, A_{Po} (Bq/L), according to the equation:

$$A_{Po} = (R - b) / F Y V$$

(d) Calculate the % of equilibrium of Po-210 and its parent Pb-210 from the equation:

$$\% E = 1 - e^{-0.693t/138}$$

(e) Calculate the Pb-210 beta activity of the sample, A_{Pb} (Bq/L), according to the equation:

$$A_{Pb} = A_{Po} / \% E$$

Where:

b = count rate of Po-210 in blank disc
b_t = count rate of Po-208 in blank disc
r = count rate of Po-210 in calibration disc
r_t = count rate of Po-208 in calibration disc
R = count rate of Po-210 in sample disc
R_t = count rate of Po-208 in sample disc
V = sample volume used in litre
t = duration of ingrowth of Po-210 in days.

4.2.5.2 Ra-226

(1) Transfer the aqueous solution left after the removal of Pb-210 Section 4.2.5,1 step 5) to a 250-mL beaker and evaporate to dryness on a hotplate.

(2) Add 50 mL of hot 10% HCl solution to the beaker and swirl to dissolve the residue.

(3) Add 3 g of potassium sulfate to the solution and stir to dissolve. Allow to cool to room temperature.

(4) Inject, rapidly, using a syringe, five, 1-mL aliquots of a 3.2% lead nitrate solution. Allow 5 minutes between aliquots. Let stand for 4 hours or more.

(5) Decant the supernatant to a clean2 50-mL beaker labelled for uranium.

(6) Transfer the white precipitate quantitatively, to a 40-mL conical centrifuge tube with the aid of deionized water.

(7) Centrifuge at 2000 rpm for 10 minutes and decant the supernatant to the uranium beaker. SAVE this solution for uranium (Section 4.2.5.4).

(8) Add 5 mL of a 0.2 M alkaline DTPA solution to the centrifuge tube, mix using a vortex mixer and heat in a hot water bath until the precipitate dissolves.

(9) Add 0.1 mL of a 1 mg/mL barium carrier solution to the DTPA solution and cool the mixture in a cold water bath.

(10) Using a syringe, inject rapidly 2 mL of the mixed sodium sulfate-acetic acid solution and mix with a vortex mixer. Allow to cool for 30 minutes. A barely visible cloudiness will be observed.

(11) Prepare a 25-mm diameter, 0.2-micron membrane filter on a filter holder with a stainless steel support screen. Place a 25 X 150 test tube inside the suction flask under the filter holder stem.

(12) Mix the contents of the centrifuge tube using the vortex mixer, pour the contents into the membrane filter under very

light vaccum and rinse the centrifuge tube with 3 mL of deionized water from a wash bottle. Collect the filtrate in the test tube and SAVE for thorium isotopes (Section 4.2.5.3).

(13) Remove the filter, glue it on a plastic disc and allow to air dry.

(14) Prepare a calibration disc by using an exact activity, A_s (Bq), of the standard Ra-226 solution and an activity of Ba-133 identical to that employed with samples at the decomposition stage (see Section C-2.4, step 3). Precipitate as barium sulfate, filter and mount in the manner described in steps 2 to 13 above. Likewise prepare a blank disc.

(15) Count blank, calibration standard and samples in the alpha spectrometer for Ra-226. Calculate count rate by dividing the number of counts by length of time counted.

(16) Count blank, calibration standard and samples on the gamma counter for Ba-133. Calculate count rate by dividing the number of counts by length of time counted.

(17) CALCULATIONS

(a) Calculate the apparent calibration constant, F, from the equation:

$$F = (r - b) / A_s$$

(b) Calculate the relative chemical yield factor, Y, the recovery of the sample disc relative to the recovery of the calibration disc, from the equation:

$$Y = (R_t - b_t) / (r_t - b_t)$$

(c) Calculate the Ra-226 alpha activity of the sample, A (Bq/L), according to the equation:

$$A = (R - b) / F Y V$$

Where:

b = count rate of Ra-226 in blank disc
b_t = count rate of Ba-133 in blank disc
r = count rate of Ra-226 in calibration disc
r_t = count rate of Ba-133 in calibration disc
R = count rate of Ra-226 in sample disc
R_t = count rate of Ba-133 in sample disc
V = sample volume used in litre.

4.2.5.3 Thorium Isotopes

(1) To the thorium isotopes fractrion (Section 4.2.5.2, step 12), add 0.1 mL of a 0.5 mg/mL cerium carrier solution and mix thoroughly using a vortex mixer.

(2) Add 2.5 mL of 10 N NaOH. Mix thoroughly using a vortex mixer and heat in a hot water bath for 15 minutes. A milky opalescence, barely visible, will be observed.

(3) Cool in a cold water bath for 15 minutes.

(4) Prepare a 25-mm diameter, 0.2-micron PVC or PTFE membrane filter on a filter holder with a stainless steel support screen.

(5) Mix the content of the test tube vigorously using a vortex mixer and pour into the filter.

(6) Rinse the test tube, filter holder and precipitate with three 5-mL portions of 80% alcohol.

(7) Remove the filter, glue it to a plastic disc and dry under a heat lamp at a distance of 10 cm.

(8) Prepare a calibration by using an exact activity, A_s (Bq), of the standard Th-228 solution and an activity of Th-234 identical to that employed with samples at the decomposition stage (see Section C-2.4, Step 3). Follow steps 1 to 7 above. Likewise prepare a blank disc.

(9) Count blank calibration standard and samples in the alpha spectrometer for Th-232, Th-230 and Th-228. Calculate their respective count rates by dividing number of counts by the length of time counted.

(10) Count blank, calibration and sampling in a beta counter for Th-234. Calculate their respective count rates.

(11) CALCULATIONS

(a) Calculate the apparent calibration factor, F, from the equation:

$$F = (r - b_8) / A_s$$

(b) Calculate the relative chemical yield factor, Y, the recovery of the sample disc relative to the recovery of the calibration disc, from the equation:

$$Y = (R_t - b_t) / (r_t - b_t)$$

(c) Calculate the Th-232, Th-230 and Th-228 activities, A_2, A_o and A_8 (Bq/L) of the sample

according to the equations:

$$A_2 = (R_2 - b_2) / F\ Y\ V$$

$$A_o = (R_o - b_o) / F\ Y\ V$$

$$A_8 = (R_8 - b_8) / F\ Y\ V$$

Where:

b_2 = count rate of Th-232 in blank disc
b_o = count rate of Th-230 in blank disc
b_8 = count rate of Th-228 in blank disc
b_t = count rate of Th-234 in blank disc
r = count rate of Th-228 in calibration disc
r_t = count rate of Th-234 in calibration disc
R_2 = count rate of Th-232 in sample disc
R_o = count rate of Th-230 in sample disc
R_8 = count rate of Th-228 in sample disc
R_t = count rate of Th-234 in sample disc
V = sample volume used in L.

4.2.5.4 Natural ranium

(1) Evaporate the uranium fraction (Section 4.2.5.2, step 7) to dryness on a hotplate.

(2) Add 2 mL of 16 N nitric acid and 30 mL of deionized water to dissolve the residue.

(3) Transfer to a 50-mL volumetric flask. Rinse beaker with 10 mL of deionized water, add to flask and make up to volume.

(4) Determine uranium concentration, C (ug/mL), by fluorometric method described elsewhere[84].

(5) Calculate uranium concentration of the milk sample, U (ug/L) from the equation:

$$U = 50 \times C / V$$

Where:

V = volume of milk sample used in litre.

4.2.5.5 Natural Thorium and Uranium by ICP-MS

(1) Using the ICP-MS technique, determine the thorium and uranium concentrations, C_{Th} and C_U (ug/L), of the decomposed milk solution (Section 4.2.5.1, step 1).

(2) Calculate thorium and uranium concentrations, Th and U (ug/L) of the milk sample using the following equations:

$$Th = 0.1 \times 25 \times C_{Th} / V$$

$$U = 0.1 \times 25 \times C_U / V$$

Where:

V = volume of milk sample used in litre.

4.3 QUALITY CONTROL

(1) The Analytical Program must be under the direct control of the Quality Control Coordinator (Q.C.C.). It will include:

(a) Two reagent blanks interspersed with samples.

(b) One duplicate of pooled sample and one spiked standard sample to be inserted by the Q.C.C.

(2) If not sufficient mother milk is available, use artificial milk:

(a) Prepare a large batch of artificial milk to last through the project. Store 250 m aliquots in deep freezer.

(b) Run six determinations on this milk to determine the background of the radionuclides of interest.

(3) Each analysis set should not exceed 25 samples.

(4) Statistical treatment of results:

(a) Duplicates to agree within 15%.

(b) Recovery of spiked standard samples to be at least 80% on each radionuclide.

(c) Results of artificial milk to agree within 15%.

5. PROPOSED FIELD STUDY PROGRAM

5.1 OBJECTIVES AND TASKS

The three primary objectives of the Phase III, field study program, are as follows:

(1) To collect data on qualitative and quantitative factors which need to be known within a study area in which the study is to be carried out.

(2) To design, test, execute and prove procedures for conducting field investigations, and

(3) To yield useful data, data analyses and conclusions regarding concentrations of radionuclides in human milk.

This program will include the following tasks:

(1) Design of participant questionnarie

(2) Identification of study sites and acquisition of participants

(3) Information kit and training

(4) Sample collection

(5) Laboratory analysis

(6) Data capture

(7) Statistical analyses

(8) Report

5.1.1 Design of Participant Quesionnaire

Knowledge of input data on qualitative and quantitative factors in this study will be needed to determine the significance of the pathway which involves transfer of radionuclides to an infant through human milk consumption. Qualitative or descriptive factors relate to the amount and distribution of radionuclides in human milk within a sampled population group by person, place and time. Qualitative or analytical factors relate to the reasons for relatively high or low milk radionuclide concentrations observed in specific population groups.

Qualitative factors for lactating mothers within each population group will include the following:

- age
- health
- residence history (i.e. duration at present residence and previous residences)
- proximity to areas of radioactivity, high natural background areas and/or uranium mining activities
- occupation, either an atomic energy worker or a non-atomic energy worker.

Quantitative factors which will have to be examined for each of the lactating mothers are as follows:

- diet and daily intake of various foods by each mother
- volume of breast milk produced daily by lactating mothers (i.e. about 0.8 L for a mother with one infant).

Qualitative factors for the infants will include age and general health. Qualitative factors for the infant receiving

breast milk will include the following:

- diet and daily milk consumption
- relative percentage of breast milk to total daily milk consumed

Previous experience suggests that a wide range of relevant information may be required in order to properly interpret results. Depending on the experimental design, it may be necessary to structure the questionnaire in two parts: one being a general one-time only questionnaire; the second being a shorter questionnaire filled out with each sample submitted by an individual. We believe that it will be desirable to have each participant contribute several samples over time in order to establish variability in concentration within individuals as well as between individuals (e.g 100 participants @ 4-6 samples each equals a total of 400-600 samples).

Collection of questionnaires would likely be conducted by self-addressed postage-paid envelopes. Follow-up would be conducted on late submissions.

5.1.2 Identification of Study Sites and Acquisition of Participants

The study design is to determine radionuclide transfer to and from human milk in areas of high ambient natural exposure and in areas of low ambient natural exposure. The latter represents areas of control population in mothers and infants. Based on the underlying premises of the study design, population bases will be identified across Canada. It may not be necessary to have these bases broadly distributed at this point in the survey program.

We believe that it may be useful to select the following population bases:

- 2 urban centres of similar size - one with high ambient natural radiation exposure, one with low ambient natural exposure.

- 2 rural centres of similar nature - one with high exposure, one with low exposure.

Within each of these four population cells, it is likely that twenty-five participants, would be required to execute the field program (total of 100 participants). Potential participants would be identified in conjunction with local medical practitioners, public health services boards, hospitals or associations (e.g. La Leche League). Participation would be solicited by way of posters, personal letters and follow-up information telephone calls. Care will be given to providing full information on the study and assurance of confidentiality.

Within urban areas, it is not likely that any incentive would be required but test results would likely have to be conveyed to participants and interpreted for them in proper medical terms. This might be done by letter or in conjunction with local medical practitioners.

Study criteria that will be used to screen potential participants include:

- permanent residence within area of interest in Canada for the last five years
- no travel outside the area of interest for seven days preceding sample collection
- ability and desire to provide four-to-six milk samples of approximately 200 ml each
- willingness to provide their milk manually

5.1.3 Information Kit and Training

In conjunction with experimental design and laboratory process specialists, an information kit for participants will be developed. The kit will contain:

- a letter of introduction
- requirements of participants
- agreement form
- local program representative's name and telephone
- detailed instructions for sample collection and storage
- pamphlet on breast feeding
- bottles and related materials
- questionnaire

The letter of introduction serves the following functions:

- introduces the study
- explains the study objecties and requirement of participation
- presents confidentiality procedures.

The agreement form obtains consent of approval by the participant. The questionnaire is the primary data collection instrument on the participant.

Before the collection begins at a site, a training session will be held to acquaint the program manager and the local program representatives. The session will address the study objectives, use of the data collection instruments, administrative instructions, quality control procedures, and collecting, packing and shipping milk samples to the designated radiochemical laboratory.

5.1.4 Sample Collection

Four to six 48 hours composite samples are required from each participant. The composite samples should be 250 ml or more. The complete sample collection procedure is detailed in Section 4.1.1.

5.1.5 Laboratory Analysis

Complete methodology is detailed in Section 4.2.

5.1.6 Data Capture

Data from questionnaires would be immediately entered into a micro-computer data base at a central location. Data would be screened in terms of internal consistency and completeness. Gaps or deficiencies would be followed up by letter or telephone.

Procedures for ensuring questionnaire response quality control would be implemented (e.g. in the question being interpreted properly by the respondent) and linkage with the physical milk sample and concentra-tion data ensured. The data base would be prepared on a conventional PC software system such as DBASE or ZIM. All data disks would be transferred to the client upon completion of the study.

Comparisons will be made between concentration data of radionuclides in milk and the characteristic factors of each mother. For example, age is usually thought to be an important factor in epidemiological studies. The relationships between concentration and age will be tabulated according to the following age groups: 15-19, 20-24, 25-29, 30-34, 35-39, 40-44 and >45.

5.1.7 Statistical Analysis

It is expected that a number of univariate and multivariate analyses will be required to properly analyse and interpret the questionnaire an laboratory results. The nature of these analyses will be determined in conjunction with the client, but likely would include one or more of the following:

- multiple correlations
- analysis of variance
- principal components

Conversely, the client may want raw statistics (e.g. means, variances, ranges) only at this stage with detailed data provided to the client on disk to support their own analyses. We have assumed for the moment that the consultant would be responsible for conductiong a moderate level of statistical analysis.

The results of these analyses would be written up and incorporated in the final report.

5.1.8 Report

A report will be submitted which will include:

- analytical data
- information from questionnaire
- statistical analyses.

5.2 SCHEDULE

It is estimated that a total of 18 to 24 months is required to complete this field study program. The schedule is divided into three periods:

(1) Period I - First 3 to 4 Months

During this period, Task I (design of participant questionnaire), Task 2 (identification of study sites and acquisition of participants) and Task 3 (information kit and training) will be conducted.

(2) Period 2 - 12 to 18 Months

Task 4 (sample collection), Task 5 (laboratory analysis) and Task 6 (data capture) will be done. This is the lengthiest period due to the following reasons. Firstly, the sample collection will likely be spread over 12 months or longer since not all participants will be at lactating stage simultaneously. Secondly, the radiometric counting time will take approximately 40 to 60 weeks. Each sample will have three alpha sources, one for each of Pb-210 (Po-210), Ra-226 and Th-230. Therefore a total of 1200 to 1600 alpha sources will require radiometric counting. Since the counting time for them is 4 hours each (see Section 3.3), hence only three sources can be counted per day per alpha spectrometer. By dedicating two alpha spectrometers to this project 10 to 15 months will be required.

(3) Period 3 - 2 Months

As soon as all laboratory analyses are completed, statistical treatment of data will proceed. A report will also be prepared.

5.2 PROGRAM COST ESTIMATE

The entire cost is estimated to be between $185,000 and $245,000. The breakdown follows:

(1) Labour $50,750

Senior consultants	45 days @ $550/day	$24,750
Junior consultants	30 days @ $400/day	12,000
Technicians	40 days @ $250/day	10,000
Secretarial	20 days @ $200/day	4,000

(2) Analytical $120,000 - $180,000

Analysis of Pb-210, Ra-226 , Th-230, nautral thorium and uranium including sample decomposition and quality control:

400 - 600 samples @ $300 each

(3) Out-of-Pocket Expenses $14,000

Travel/accommodation	$5,000
Telephone	2,000
Courier	5,000
Printing	1,500
Miscellaneous	500

This cost estimate does not include the salary, if required, for the four local program representatives.

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